II. "A New Test for Albumin and other Proteids." By JOHN A. MACWILLIAM, M.D., Professor of the Institutes of Medicine in the University of Aberdeen. Communicated by Sir WM. ROBERTS, F.R.S. Received March 5, 1891.

Salicyl-sulphonic acid is a remarkably powerful precipitant of proteid substances; it is an extremely delicate reagent for the detection of proteids in solution; it acts upon all the classes of proteid bodies.

I shall state the results I have obtained with this reagent under two heads:—

I. Its action on the various classes of proteids.
II. Its use as a test for the presence of proteids in urine.

I. *The Action of Salicyl-sulphonic Acid on the various Classes of Proteids.*

In order to obtain the full effect of this reagent, it should be used in *saturated* watery solution, and a drop or two of this solution should be added to a small amount (*e.g.*, 1 or 2 c.c.) of the fluid to be tested, and the test-tube should be shaken so as to mix its contents well. When any considerable amount of proteid is present, a copious white precipitate at once results; with only minute amounts of proteid a cloudiness or opalescence of the fluid is what occurs. This cloudiness or opalescence is uniformly diffused over the fluid. When dealing with traces of proteids it is well to use a control tube containing some of the fluid to be tested, and if dilution has been performed, another control tube with some of the water (or other liquid) used for dilution along with one or two drops of the salicyl-sulphonic acid. The observer then holds the three tubes between him and the light, and looks through them at a dark ground. It is only, however, when dealing with very slight traces of proteids that these precautions are at all necessary.

A. *Native Albumins.*

(*a.*) *Egg Albumin.*—Upon this proteid salicyl-sulphonic acid acts with much precision. When a solution is obtained by diluting white of egg with water in the proportion of 1 part white of egg in 20 parts of the mixture, the addition of the reagent causes a dense white precipitate to be at once formed. On boiling, the precipitate becomes markedly flocculent. (The solution of white of egg of course contains globulin, but when this is removed by saturation with magnesium sulphate the albuminous filtrate gives the same reaction with salicyl-sulphonic acid as the original fluid.)

When a similar solution is made with white of egg and the proteid removed by thorough saturation with ammonium sulphate (after slight acidulation) and filtration, the proteid-free filtrate gives no precipitate with salicyl-sulphonic acid. But if some of the precipitate thrown down by the ammonium sulphate, after being washed with a saturated solution of the salt, be redissolved in water, the solution of proteid so made gives a copious precipitate when tested as before. (When considerable amounts of ammonium or magnesium sulphate are present, a few crystals may form and sink to the bottom of the tube, apart from the presence of any albumin. This, however, does not at all interfere with the working of the test, as such crystals, floating in the fluid at first and then sinking to the bottom, bear no resemblance to the uniform turbidity or opalescence dependent on the presence of albumin. These salts are easily removed by dialysis.)

Similarly, when the white of egg solution is treated with a large excess of absolute alcohol (after slight acidulation with acetic acid), so as to precipitate all the proteids, and is then filtered, the filtrate shows no precipitate when tested with salicyl-sulphonic acid; removal of the alcohol from the filtrate does not influence the result. On the other hand, the alcoholic precipitate, when (after being washed with absolute alcohol) it is redissolved in water and tested, gives a striking reaction: a large amount of proteid is at once thrown down.

With very dilute solutions made with white of egg, I have compared the delicacy of the action of salicyl-sulphonic acid as a test for proteids with a number of other reagents more or less commonly employed, and I have found the former to be by far the most delicate and precise of all.

The white of egg solution was successively diluted to various degrees—1 part of the white of egg solution (1 in 20) in 100, 200, 300, 400, 600, 620, 900, and 1000 parts of water or of $\frac{3}{4}$ per cent. sodic chloride solution.

With the first degree of dilution (1 part of the 1 in 20 white of egg solution in 100 parts of water or salt solution) the following results were obtained:—

Boiling after faint acidulation with acetic acid = no reaction.
Xantho-proteic test = slight reaction.
The cold nitric acid test (Heller's) = slight reaction.
Mercuro-potassic iodide = haziness of the fluid.
Salicyl-sulphonic acid = marked cloudiness.

With the second degree of dilution (1 part of the white of egg solution in 200 parts of water):—

Boiling after faint acidulation with acetic acid = no reaction.
Xantho-proteic test = no reaction.

Mercuro-potassic iodide = doubtful haze.
Salicyl-sulphonic acid = marked cloudiness.

When the white of egg solution is diluted 400 times:—

Acidulation with acetic acid and heat = no reaction.
Xantho-proteic test = no reaction.
Heller's test = no reaction.
Mercuro-potassic iodide = no reaction.
Salicyl-sulphonic acid = distinct cloudiness.

When the degree of dilution is increased to 600 or 620 times, there is still a distinct reaction with salicyl-sulphonic acid when the test-tube is compared with control tubes containing—(*a*) the dilute solution alone, and (*b*) water with salicyl-sulphonic acid.

And even with still higher grades of dilution (900 and 1000 times) there is still an appreciable effect recognisable a little time after the addition of the reagent.

The amount of proteid present in those dilute solutions is exceedingly small.

Taking the percentage of proteid (albumin and globulin) in white of egg as 12·2, the strength of the original white of egg solution (1 in 20) would be less than 1 in 160.

When this solution is diluted 400 times, the proportion of proteid is less than 1 in 64,000; when diluted 620 times, about 1 in 100,000; and when diluted 1000 times, proteid is present only in the very minute amount of 1 in 160,000.

(*b.*) *Serum Albumin.*—Solutions containing serum albumin were obtained by saturating serum with magnesium sulphate and then filtering so as to remove the globulin; the filtrate contained the serum albumin. This fluid, when tested with salicyl-sulphonic acid, gave an abundant precipitate.

Again, when the serum is deprived of all its proteids by complete saturation with ammonium sulphate and subsequent filtration, it then fails to give the slightest sign of precipitation on the addition of salicyl-sulphonic acid. On the other hand, the proteid precipitate thrown down by ammonium sulphate, when redissolved in water, gives a dense precipitate with salicyl-sulphonic acid.

Similarly, when serum is deprived of its proteids by means of alcohol, the remaining constituents are entirely unable to give the characteristic reaction with salicyl-sulphonic acid.

The delicacy of the action of salicyl-sulphonic acid as a test for minute amounts of the serum proteids is very striking, just as in the case of the egg proteids.

The following reactions will serve as an illustration of this:—

Some ox serum was diluted with $\frac{3}{4}$ per cent. of salt solution to the extent of 1 part of serum in 1000 parts.

With this dilute fluid various tests were tried :—

Salicyl-sulphonic acid = marked opalescence at once.
Boiling after faint acidulation with acetic acid = no reaction.
Heller's test = no reaction at once. Distinct film at junction of the two fluids in a few minutes.
Xantho-proteic test = no appreciable results.
Mercuro-potassic iodide = marked cloudiness.
Saturated salt solution with hydrochloric acid (Roberts' test) = marked cloudiness.

Serum diluted to 1 *in* 10,000 :—

Salicyl-sulphonic acid = distinct cloudiness (especially after $\frac{1}{2}$—1 minute), recognisable on comparison with the control tubes in a suitable light.
Boiling after faint acidulation = no reaction.
Xantho-proteic test = no reaction.
Mercuro-potassic iodide = no reaction.
Roberts' test = no reaction.
Copper sulphate and caustic potash (Piotrowski's) = no reaction.
Heller's test = no reaction at the time nor twenty minutes afterwards.

The amount of proteid present in these dilute solutions is, approximately, as follows :—

Taking the percentage of total proteids in ox serum as 7·5,* the serum diluted to the degree of 1 in 1000 would contain less than 1 part of proteid in 13,000 ; while with the dilution of 1 in 10,000 the amount of proteid would be about 1 in 130,000.

B. *Derived Albumins.*

(*a.*) *Acid Albumin.*—A solution of acid albumin, obtained from a solution of white of egg by the addition of a few drops of a dilute acid and subsequent warming, gives a copious precipitate on the addition of salicyl-sulphonic acid.

(*b.*) *Alkali Albumin.*—A solution of this proteid, obtained by treating the white of egg solution with a dilute alkali, also yields an abundant precipitate on being tested with salicyl-sulphonic acid.

C. *Globulin.*

A solution of globulin obtained from blood serum (by precipitating with magnesium sulphate, and subsequently redissolving in dilute salt solution) gives results similar to albumin.

* Hammarsten, "Ueber das Paraglobulin," 'Pflüger's Archiv,' 1878.

And vegetable globulin obtained from flour (by extracting with 10 per cent. salt solution) behaves similarly.

D. *Fibrin.*

Solutions of fibrin, both when a dilute alkali and when 10 per cent. salt solution are used as the solvents, give white precipitates with salicyl-sulphonic acid.

In the case of all the foregoing proteids (A, B, C, and D) the precipitate does not redissolve on heating; on the other hand it becomes markedly flocculent.

E. *Proteoses.*

Primary albumoses (proto-albumose and hetero-albumose) were prepared from Witte's peptone by precipitating them with sodic chloride and (after washing with saturated solution of salt) redissolving the precipitate (containing some salt) by the addition of water. The solution so obtained gave a marked precipitate with salicyl-sulphonic acid; but in this case the precipitate redissolved on heating and reappeared on cooling.

Solutions of secondary albumose (deutero-albumose) gave similar results.

F. *Peptone.*

Solutions of peptone were obtained from albumin artificially digested with pepsin and hydrochloric acid, by saturation with ammonium sulphate and subsequent filtration. The filtrate contained peptone; it gave no precipitate with nitric acid, while it gave the xantho-proteic and the biuret reactions.

On adding a drop of salicyl-sulphonic acid to a small amount of the solution containing peptone, a precipitate was at once formed. This, like the precipitate of albumoses, readily disappeared on heating and reappeared on cooling.

Solutions containing peptone were also prepared by saturating the artificially digested albumin solution with sodio-magnesic sulphate, and similar results were obtained.

Solutions of Witte's peptone were (after being faintly acidulated with acetic acid) saturated with ammonium sulphate in some cases, and with sodio-magnesic sulphate in others. The filtrate contained peptone, the other proteids having been precipitated by saturation with the salts named and removed by filtration. The peptone solution yielded, on being tested with saturated solution of salicyl-sulphonic acid, a reaction similar to that described above, a precipitate which disappears on heating and reappears on cooling.

When the peptone was removed by precipitation with excess of alcohol and filtration, the remaining fluid failed to give the slightest proteid reaction with salicyl-sulphonic acid.

It will be noticed that there is an important difference in the behaviour of proteoses and peptones as compared with the other proteid bodies under the influence of the salicyl-sulphonic acid; the precipitate yielded by the proteoses and peptones clears up on heating, and reappears on cooling, while the precipitate of the other proteids does not clear up on heating, but, on the other hand, becomes markedly flocculent. In this respect the reagent resembles picric acid and mercuro-potassic iodide, and to some extent also nitric acid. It differs from the latter, however, inasmuch as the latter gives no precipitate with peptones.

As regards the nature of the precipitate of egg albumin, or serum albumin, thrown down by salicyl-sulphonic acid, its general appearance might suggest that not only precipitation, but also coagulation, had occurred, as with nitric acid, &c. But the fact that it is soluble on the addition of a sufficiently large amount of a very weak solution of potassic hydrate (0·1 per cent.) or of sodium carbonate (1 per cent.) shows that no coagulation could have taken place. Solution of the precipitate does not take place as long as any acidity remains in the fluid. And when it has been redissolved the addition of a very small amount of a weak acid (nitric, acetic, sulphuric) can again bring about precipitation.

The precipitate of albumin thrown down by salicyl-sulphonic acid is not redissolved by the addition of even considerable amounts of this acid, nor is it dissolved by nitric acid, except when a large amount of the strong acid is added.

When salicyl-sulphonic acid is made to act upon albumin for some time, especially at a high temperature, and the precipitate is then filtered off, the filtrate shows a very marked coloration, varying from a pinkish tint to a bright amethyst. The filter paper commonly shows a staining of the same colour. When the fluid is filtered hot, the filtrate usually shows evidence of containing albumoses; it becomes turbid on cooling, and clears up on heating. The coloration of the filtrate is most marked and pure when the fluid is clear (*e.g.*, when hot); when it is turbid the colour is, to some extent, masked and modified (often to an orange-pink tint) by the presence of the precipitate.

II. *On the use of Salicyl-sulphonic Acid as a Test for Proteids in Urine.*

Salicyl-sulphonic acid gives no precipitate whatever with normal urine.

In the case of albuminous urine, on the other hand, it constitutes an extremely delicate and precise test for the presence of proteids. By its use, coupled with heat, one can, with great facility, recognise very minute amounts of proteid substance, and can discriminate between the so-called "albumin" (albumin and globulin), most commonly found, and the albumoses or peptones, if such are present. This is easy, since the application of heat in the latter case causes the precipitate to disappear—to reappear on cooling; while, in the case of ordinary albuminous urine, the precipitate does not clear up on heating.

The method of performing the test in the case of urine is the same as with other solutions.

A very small amount of urine should be taken in the bottom of an ordinary test-tube (*e.g.*, half an inch, or so), or a very small, narrow test-tube may be used. The acid must be in a thoroughly saturated aqueous solution. A drop of this solution is then added to the urine, and the tube is shaken. When any considerable amount of proteid is present, a copious precipitation immediately occurs; when there is only a minute proportion of proteid, the fluid becomes uniformly opalescent.

The delicacy of the test is shown by the following results, obtained with one of the samples of albuminous urine examined :—

The urine was diluted to 10, 20, 30, 40, 50 times. With the weakest of these fluids (1 in 50), salicyl-sulphonic acid quickly gave a marked opalescence.

Heller's test gave no reaction for a considerable length of time; then it gave a doubtful haziness at the junction of the nitric acid and the urine.

Picric acid (saturated watery solution) = no reaction.

Cupric sulphate and potassic hydrate = ,, ,,

The urine was then diluted twice as much, to 100 times, and still gave, after standing a little, a distinct cloudiness with salicyl-sulphonic acid, specially noticeable when the tube was compared (in a suitable light) with two control tubes, containing respectively (*a*) some of the dilute urine, and (*b*) water with a drop or two of salicyl-sulphonic acid.

Even with much greater degrees of dilution, appreciable results were got by means of this test. Without going to the extreme limits of its application, however, I find that in the case of the urine diluted 100 times, the amount of albumin contained must have been exceedingly small. A quantitative determination showed that the amount of urine present in the *undiluted* urine was about 0·1 per cent. Hence the amount present in the urine diluted 100 times must have been about 1 in 100,000.

The opalescence caused by the addition of salicyl-sulphonic acid to

such very dilute albuminous solutions does not clear up on boiling. It remains persistent for days in the cold, the precipitate after a time assuming the form of a marked cloud at the lower part of the test-tube.

The effect of adding salicyl-sulphonic acid to samples of albuminous urine from which the albumin had been removed was tested in many cases, and always with negative results. The albumin was precipitated by means of absolute alcohol (after acidulation, when necessary) or by saturation with ammonium sulphate. The proteid-free filtrate gave not the slightest reaction in any instance when tested with salicyl-sulphonic acid in the usual way.

The characteristic reaction of even minute amounts of albumin in the urine I found to be given, on the addition of salicyl-sulphonic acid, in very numerous and various conditions—in acid, neutral, and alkaline urine; in urines rich in mucin, phosphates, urates, &c.; in urines containing bile, sugar, and other abnormal constituents. My results, obtained from the examination of a large number of samples of urine, have not indicated that the applicability of the test is complicated or interfered with by any of the abnormal constituents present. The urine of persons under the influence of various drugs (*e.g.*, alcohol, quinine, sulphonal, croton-chloral, iodide of potassium, chloroform, salicylate of soda, strophanthus, &c.) has not shown the slightest reaction with salicyl-sulphonic acid when shown to be free from albumin by other tests (after concentration) or when freed from albumin, if such has been present, by means of alcohol or ammonium sulphate.

I have also examined the effect of salicyl-sulphonic acid upon solutions of various substances, many of which give precipitates with certain of the well-known reagents for the detection of albumin in urine—solutions of strychnine, digitalin, morphia, nicotin, chloral hydrate, atropine sulphate, aconitine, ergotin, caffein citrate, strophanthin, sulphonal, gallic acid, quinine, bromide of potassium, copaiba, &c.—and in no case have I seen any reaction at all resembling that yielded by proteids.

The conclusion to which my results up to the present lead is that salicyl-sulphonic acid is probably the most delicate and precise of all known reagents for the detection of proteids in solution.

Note on the Nature of Salicyl-sulphonic Acid.

Salicyl-sulphonic acid is a whitish crystalline substance, readily soluble in water and in alcohol. On slow evaporation of its aqueous solution, it crystallises in long, thin needles.

Its formula and formation are stated in Beilstein's 'Handbuch d. org. Chemie,' 2nd ed., vol. 2, p. 969. (For this reference I am in-

debted to Professor Japp, F.R.S.) The formula is there given as $C_6H_3(OH)(SO_3H)COOH$; and its formation by the action of sulphuric anhydride on salicylic acid (Mendius, 'Ann. Chem. Pharm,' vol. 103, p. 45), or by heating salicylic acid with concentrated sulphuric acid (Remsen, *ibid.*, vol. 179, p. 107). It is said to be very stable, and to undergo no change on heating with nitric acid.

The specimens of salicyl-sulphonic acid which I have used in my experiments were obtained from Messrs. Davidson and Kay, Union Street, Aberdeen.

III. "The Influence of Oxygen on the Formation of Ptomaines." By William Hunter, M.D., F.R.S.E. Communicated by Professor M. Foster, Sec. R.S. Received March 11, 1891.

(Abstract.)

A special interest attaches to the *rôle* of oxygen in the life-history of bacteria. Very wide differences exist, however, between different groups in respect of its importance. To the great majority a free supply of oxygen is absolutely essential for their proper growth and development; to a small minority the converse applies, growth proceeding best in the absence of oxygen, if indeed it is not entirely prevented by its presence; while, lastly, in the case of an intermediate group it seems almost immaterial whether oxygen be present or not, growth proceeding apparently equally well in both conditions, provided that the supply of food be otherwise suitable.

Of these three groups of "obligate aërobic," "obligate anaërobic" and "facultative aërobic" bacteria, respectively, the last has perhaps the greatest interest for the pathologist, as it is to it that the great majority of pathogenic organisms belong.

The question is thus an interesting one, to what extent the pathogenic properties of this class of bacteria are related to the power they, apparently under necessity, possess of obtaining their supply of oxygen from the food constituents themselves when the supply in the air is cut off.

The present paper deals with the results of an investigation undertaken in this relation.

It was necessary that the class of bacteria selected for study should be one whose pathogenic properties were not constant, but subject to variations presumably connected with the character of their surroundings.

The bacteria of ordinary putrefaction possess in a special degree this qualification, their chemical products differing much in character and poisonous action under different, for the most part as yet unknown, conditions.